# A COMPARISON OF METHODS FOR THE DISPERSION OF CULTURES OF BENTHIC DIATOMS

Domenico VOLTOLINA

Centro de investigación Científica y de Educación Superior de Ensenada (C.I.C.E.S.E.), Depto. de Acuicultura, Apdo. Postal 2732, Ensenada, Baja California, México.

ABSTRACT - The results of a series of tests demonstrated that cultures of benthic diatoms can be successfully and evenly dispersed using low frequency ultrasound. The method has the advantage, over others tested, of causing minimum damage to the organisms in culture.

RÉSUMÉ - Les résultats d'une série de tests ont démontré que les cultures de diatomées benthiques peuvent être dispersées avec succès et de façon uniforme par l'usage d'ultrasons à basse fréquence. Cette méthode a l'avantage, sur les autres méthodes testées, de causer le minimum de dommages aux organismes en culture. (traduit par la rédaction)

KEY WORDS : benthic diatoms, culture, dispersing techniques.

## INTRODUCTION

In the course of some studies on the responses of the common microfouler *Amphora coffeaeformis* Agardh to the toxic principles of several antifouling paints (e.g. Robinson *et al.*, 1985; Brown *et al.*, 1988) it was noted that the variability of the data was largely due to the improper biomass evaluation, originated either by incomplete detachment of the culture from the experimental vessel, or by contagious cell distribution in the samples. This problem seems to have been often overlooked in benthic diatoms work: even in methodological manuals (e.g. Guillard, 1973) dispersing techniques are only mentioned, without any further indication or warning.

This note compares the results obtained with some recovery and dispersing techniques, focusing on their effectiveness and on the possibility of causing physiological damage to *Amphora coffeaeformis*. The method chosen was later found satisfactory, with slight modifications as to time of treatment, for several other benthic species (Voltolina, 1990), and is now currently used in our laboratory even for delicate plankton species, as the most effective and least destructive dispersing technique.

Source : MNHN, Paris

## METHODS

Cultures were grown at 10 ± 1°C in 125ml Erlenmeyer flasks with 75ml of ES medium (MacLachlan, 1973) under a constant illumination of 300$\mu$E m$^{-2}$ s$^{-1}$. All tests were run with cultures of various ages, from five to approximately thirty days. The methods of culture detachments and suspension tested were:

- Manual shaking with 20g of glass beads (diameter: 2mm). Shaking lasted until visual observation suggested that total detachment and suspension had been achieved, or for a minimum of two minutes.

- Manual detachment with a soft rubber scraper, followed by manual or mechanical (vortex mixer at maximum speed) shaking for the same times as in the previous method.

- Sonication with a jeweller's ultrasonic cleaner (L&R Maxomatic: output 45W at 80-90 KHz). Sonication times were from two to ten minutes, with intermediate stops at three and five minutes.

Biomass was measured as *in vivo* fluorescence with a Turner 111 fluorimeter, and is given in the text and in Table I as arbitrary fluorescence units. For sake of simplicity, experimental procedure are given in the text, together with the results obtained in each type of experiment.

## RESULTS AND DISCUSSION

Recovery from culture vessels: ten cultures were removed from the flasks with each of the two manual treatments, which was repeated twice in each case; after this, 75ml of fresh medium were added to the flasks, and these were sonicated for increasing lengths of time.

Fluorescence readings indicated that, on average, 17% of the biomass was left adhering to the walls if glass beads were used, and 6.5% when the culture was detached using a rubber scraper. Further, they showed that three minutes of sonication were sufficient for an almost complete removal of the remaining biomass (17.4 ± 4.4 and 6.1 ± 3.3 as compared to 17.7 ± 4.5 and 6.5 ± 3.2 after ten minutes respectively). Both methods resulted, in addition, fairly inconsistent, with ranges of biomass recovered varying between 10.8 and 23.3 with the first method, and between 1.9 and 12.0 with the second.

After sonication, each flask was left standing overnight in a refrigerator with 25ml of 90% acetone. No pigments were measurable fluorimetrically in the resulting extracts, proving that sonication had achieved complete detachment of the cultures.

Culture dispersion: ten cultures were used for each method. After treatment, ten replicate *in vivo* fluorimetric readings were taken on each of an equal number of samples, and mean fluorescence and index of dispersion ($I = s^2/x$) calculated for each culture. Table I shows that shaking with glass beads yielded a random distribution only in five out of ten cases, and that scraping and shaking, both manually and mechanically, were only partially

Source : MNHN, Paris

| Treatment | Dispersion Index | | Distribution | | |
|---|---|---|---|---|---|
| | Range | I | Cont. | Poiss. | Reg. |
| Glass Beads | 0.67- 3.38 | 2.07 | [illegible] | 5 | 0 |
| Scraping-hand shaking | 0.09-10.36 | 1.56 | 2 | 2 | 6 |
| " -mech. shaking | 0.14- 8.99 | 1.41 | 2 | 2 | [illegible] |
| Sonication-2 minutes | 0.05- 0.32 | 0.17 | 0 | 0 | 10 |
| " 3 minutes | 0.02- 0.08 | 0.05 | 0 | 0 | 10 |
| " 5 minutes | 0.01- 0.07 | 0.03 | [illegible] | 0 | 10 |
| " 10 minutes | 0.01- 0.06 | 0.03 | [illegible] | [illegible] | 10 |

Table I - Range and mean value of the dispersion index $I = s^2/x$, and number of cultures showing a contagious, Poisson or regular diatom distribution, as measured with ten successive *in vivo* fluorescence readings for each of ten cultures, after the treatment specified. I values higher than 1.880 or lower than 0.369 indicate contagion or regularity respectively ($P < 0.05$).

| | H.S. | | Son. | |
|---|---|---|---|---|
| | x | $s^2$ | [illegible] | $s^2$ |
| Culture 1 | 289.2 | 7956.6 | 257.0 | 2872.8 |
| | 242.3 | 6626.0 | 226.9 | 2190.2 |
| | 218.7 | 14328.1 | 221.1 | 2704.0 |
| | 74.2 | 479.6 | 204.0 | 2052.1 |
| Culture 2 | 794.5 | 35118.8 | 639.3 | 5610.0 |
| | 641.2 | 16770.3 | 611.4 | 4651.2 |
| | 449.7 | 12611.3 | 601.9 | 2470.1 |
| | 427.9 | 14328.1 | 584.7 | 5299.7 |
| Culture 3 | 444.7 | 9900.3 | 337.5 | 2440.4 |
| | 301.6 | 4160.3 | 331.2 | 3317.8 |
| | 227.9 | 7638.8 | 330.9 | 3192.3 |
| | 87.1 | 317.7 | 317.7 | 1738.9 |
| Culture 4 | 111.3 | 590.5 | 84.5 | 136.9 |
| | 79.2 | 259.9 | 83.9 | 198.8 |
| | 77.9 | 388.1 | 82.6 | 193.2 |
| | 70.5 | 272.3 | 75.3 | 75.7 |
| Culture 5 | 185.0 | 3294.8 | 194.2 | 1069.3 |
| | 179.1 | 3375.6 | 191.0 | 2237.3 |
| | 168.1 | 3003.0 | 186.4 | 585.6 |
| | 161.2 | 1901.0 | 180.2 | 1122.3 |

Table II. - Cell numbers (as mean of three replicate counts on a $9 mm^2$ haemacytometric grid), variances and comparison of means (Duncan's multiple range test, $\alpha = 0.05$), as obtained from four different samples taken from five *Amphora coffeaeformis* cultures. H.S. = hand shaken; Son.: sonicated.

effective in obtaining an even suspension. With sonication, the values of the dispersion indices were lower and evenness was achieved in all cases.

Source : MNHN, Paris

The variability of haemacytometric counts was also studied: while samples from cultures dispersed manually showed occasionally significant differences, this did not happen when the same sample was sonicated for five minutes (Table II).

Cell damage: the possibility of causing cell damage with the ultrasound treatment was tested by measuring the photosynthetic activity of ten cultures before sonication and one hour after being sonicated for five minutes, the above interval being necessary for *Amphora* to settle and adhere again to the glass walls. No difference was evident between measurements, since oxygen evolutions, expressed as p.p.m. evolved by $10^3$ fluorimetric units, were 1.87 $\pm$ 0.36 and 1.95 $\pm$ 0.61 p.p.m for the first and second half hour of measurement before sonication, and 1.90 $\pm$ 0.44 and 2.31 $\pm$ 0.37 p.p.m for the same intervals, after ultrasound treatment. The one way analysis of variance gave column and mean squares values of 0.43 and 0.21 respectively, with a calculated F ratio of 2.05 as compared to the tabulated 2.88 F value for $\alpha$ = 0.05. These results demonstrate that sonication is a safe, nondestructive method, for the detachment and suspension of this diatom.

## CONCLUSION

Ultrasound treatment is a safe and efficient method for the suspension of benthic diatoms; it has later been found effective in breaking cell clumps of plankton diatoms such as *Skeletonema costatum* Cleve and *S. menzelii* Guillard *et al.* For these however, as well as for other weakly silicified diatoms, lengthy treatments should be avoided, two-three minutes being the limit after which evidence of cell damage has at times been noticed.

Care should also be taken in instrument selection. Several ultrasonic cleaners are now on the market: the author's experience with other models has been always satisfactory, provided that the frequency of output is in the 75 to 100 KHz range. Below the lower value the instrument's effectiveness is poor, while higher frequencies have proven damaging to the cultures.

## ACKNOWLEDGMENTS

Part of this work was carried out at Royal Roads Military College, Victoria (Canada) while the author was the recipient of a N.S.E.R.C. Visiting Scientist Fellowship supported by funds from the Department of National Defense (D.N.D.-C.R.A.D.) of Canada, and at the Division of Life Sciences, Scarborough Campus, of the University of Toronto.

## REFERENCES

BROWN L.N., ROBINSON M.G. & HALL B.D., 1988 - Mechanisms for copper tolerance in *Amphora coffeaeformis* - internal and external binding. *Mar. Biol.* 97: 581-586.

Source : MNHN, Paris

GUILLARD R.R.L., 1973 - Growth measurements: division rates. *In* STEIN J.R. (Ed.), *Handbook of phycological methods. Culture methods and growth measurements.* Cambridge University Press, Cambridge, London, New York, pp. 289-311.

MacLACHLAN J., 1973 - Isolation and purification: growth media-marine. *In* STEIN J.R. (Ed.) *Handbook of phycological methods. Culture methods and growth measurements.* Cambridge University Press, Cambridge, London, New York, pp. 25-51.

ROBINSON M.G., HALL B.D. & VOLTOLINA D., 1985 - Slime films on antifouling paints. Short-term indicators of long-term effectiveness. *J. Coating Technol.* 57:35-41.

VOLTOLINA D., 1990 - Producción semicontinua de microalgas bentónicas en un criadero de abulón. *In* DE LA LANZA-ESPINO G. & ARREDONDO-FIGUEROA J.L.(Eds.), *La acuicultura en México: de los conceptos a la producción.* Instituto de Biologia, U.N.A.M., México, 316 p.

Source : MNHN, Paris